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Dystrophin in Frameshift Deletion Patients with Becker Muscular Dystrophy

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Summary

In a previous study we identified 14 cases with Duchenne muscular dystrophy (DMD) or its milder variant, Becker muscular dystrophy (BMD), with a deletion of exons 3–7, a deletion that would be expected to shift the translational reading frame of the mRNA and give a severe phenotype. We have examined dystrophin and its mRNA from muscle biopsies of seven cases with either mild or intermediate phenotypes. In all cases we detected slightly lower-molecular-weight dystrophin in 12%–15% abundance relative to the normal. By sequencing amplified mRNA we have found that exon 2 is spliced to exon 8, a splice that produces a frameshifted mRNA, and have found no evidence for alternative splicing that might be involved in restoration of dystrophin mRNA reading frame in the patients with a mild phenotype. Other transcriptional and posttranscriptional mechanisms such as cryptic promoter, ribosomal frameshifting, and reinitiation are suggested that might play some role in restoring the reading frame.

Introduction

Duchenne muscular dystrophy (DMD) and its milder variant, Becker muscular dystrophy (BMD), are allelic X-linked disorders caused by mutations in the gene coding for dystrophin, a 427-kD cytoskeletal protein (Worton and Thompson 1988). DMD is a rapidly progressive disease in which the affected boys lose the ability to walk independently before the age of 12 years. BMD has a slower rate of progression, affected boys remaining ambulant beyond the age of 16 years, with a few leading near-normal lives. Those who lose independent ambulation between the ages of 12 and 16 years are classified as "intermediates" (Dubowitz 1978, 1989; Emery 1987).

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Approximately 60% of individuals affected with DMD and BMD have a deletion of one or more exons of the dystrophin gene, but no correlation has been found between the site or the extent of deletion and the severity of the disease (den Dunnen et al. 1989; Gillard et al. 1989; Koenig et al. 1989). In the majority of cases, deletions that maintain the translational reading frame of mRNA result in a mild phenotype presumably due to the production of a shorter but partially functional dystrophin molecule. In contrast, deletions that shift the translational reading frame of mRNA usually result in a severe phenotype (DMD), consistent with production of inactive truncated protein (Monaco et al. 1988; Bulman et al. 1991*b*). There are, however, a number of notable exceptions to this reading-frame rule. In our earlier study of deletions confined to the first 10 exons of the gene (Malhotra et al. 1988), we identified three severe (DMD), six mild (BMD), and five intermediate cases, all with a deletion of exons 3–7, a deletion predicted to shift the reading frame. In several studies of deletions throughout the dystrophin gene (Baumbach et al. 1989; den Dunnen et al. 1989; Gillard et al. 1989; Koenig et al. 1989)

approximately 6% of the patients have now been identified as exceptions to the reading-frame rule. In the present report, an extensive analysis of dystrophin and its mRNA has been carried out for seven patients with a deletion of exons 3–7.

Material and Methods

Patients

Seven affected males examined in this study are described in table 1. Individuals classified as BMD patients include H1456, H3924, and UHH.DF. H1456 is a 37-year-old male who lost ambulation at age 33 years, and H3924 is 22 years old and still ambulant. UHH.DF is a 6-year-old familial BMD case who presented at age 3 years with proximal muscle weakness but who can still run and jump. His classification was based on his uncle with classic BMD, who could walk independently at the age of 24 years. H3975, an unusual case of BMD (?BMD), presented at age 5 years with myoglobulinuria, complained of fatigue, but had no overt clinical muscle weakness. He died at age 7 years of rhabdomyolysis. His prognosis indicated that he would have remained ambulant beyond the age of 12 years. H4058 is a 26-year-old intermediate patient who presented at the age of 8 years. He was provided with braces at age 12½ years and was ambulant to the age of 20 years, an age well outside the usual DMD range. Patients too young to be classified as either intermediate or BMD are classified here as intermediate/BMD and include H4049 and UHS1323. H4049 is a 15-year-old patient who presented at the age of 7½ years and is still ambulant. Case UHS1323 is a 13-year-old male who presented at the age 7½ years. His disease progression is very slow, similar to that of BMD.

Case numbers with the prefix "H" are from Hammersmith Hospital, London; "UHS" is from the University Hospital, Saskatchewan; and "UHH" is from the University Hospital, Hershey, PA. In a previous study, cases H1456, H4049, H4058, and UHS1323 have been referred to as "Guys1456," "Guys1587," "Guys991," and "HSC1323," respectively (Malhotra et al. 1988).

Antibody Production

The anti-dystrophin antisera P6, recognizing amino acids 2814–3028, and 1461, recognizing the last 17 amino acids of dystrophin (amino acids 3667–3685), were raised in rabbits. Production, purification, and characterization of antisera 1461 and P6 have been described in detail (Zubrzycka-Gaarn et al. 1991; T. G. Sherratt and P. N. Strong, unpublished data). Antiserum 9219, recognizing amino acids 67–667 from exons 4–16, was raised in sheep (Bulman et al. 1991*a*). The rabbit anti-60K dystrophin antibodies (amino acids 407–815) were a gift to one of us (P.N.S.) from Dr. L. Kunkel, Boston (Hoffman et al. 1987).

Western Blot and Immunocytochemical Analysis

Western blot analysis and the densitometric quantitation were carried out using P6, anti-60K, and 1461 antibodies according to a method described elsewhere (Patel et al. 1988). Dilutions for the primary antibodies were 1:3,000 for P6 and 1:1,000 for the anti-60K and 1461 antibodies. A peroxidase-conjugated donkey anti-rabbit antibody was used for the detection of bound P6 and anti-60 K antidystrophin antibodies, and an alkaline phosphatase–conjugated goat anti-rabbit secondary antibody was used to detect bound 1461 antibody. For immunocytochemical analyses, 5–7-mm-thick transverse cryostat sections were pre-

Table 1

A Summary of Phenotypes of Patients Examined in Present Study

Patient	Diagnosis	Age at Biopsy (years)	Age at Loss of Ambulation (years)
H1456	BMD	36	33
H3924	BMD	21	Ambulant at age 22 years
H3975	?BMD	7	. . .
H4058	Intermediate	25	12.5
H4049	Intermediate/BMD	14	Ambulant at age 15 years
UHH.DF	BMD	4	Ambulant at age 6 years
UHS1323	Intermediate/BMD	12	Ambulant at age 13 years

pared from frozen muscle biopsies. Sections were immunostained with antibodies 1461 and 9219 at dilutions 1:40 and 1:500, respectively, and the bound antibodies were detected with a horseradish peroxidase system according to a method described elsewhere (Zubrzycka-Gaarn et al. 1988). The sections were counterstained with hematoxylin to visualize the morphological features.

Southern Blot Analysis

DNA (5 μg) was digested with *Eco*RI, *Hin*dIII, and *Bam*HI restriction endonucleases, was separated on 0.7% agarose gels, and was transferred to Hybond-N membrane. The membranes were hybridized with ^{32}P-dCTP random-primed DNA probes. The intron 7 probes used for the analysis included XJ (DXS208)–region probes XJ1.1, 1.2, 2.1, 2.3, 3.1, 4.1, 5.1, 6.1, 6.2, 7.1, and 7.2 (Ray et al. 1985; Thompson et al. 1986; Malhotra et al. 1988).

cDNA Amplification and Sequencing

The forward (F) and reverse (R) oligonucleotide primers identical and complementary to mRNA sequences were 1F (exon 1), 5′-GACGAATTCGGTGGGAAGAAGTAGAGGACTG-3′; 9R (exon 9), 5′-GACGAATTCGGCTTAGGGGAAGAAGTTCTCTCA-3′; 10R (exon 10), 5′-CACTCTCCATCAATGAACTG-3′; 15R (exon 15), 5′-GACGAATTCTGTGAATCTTGTTCACTGCATC-3′; and 16R (exon 16), 5′-ATACAGTTTGCCCATGGATTGC-3′. The underlined sequence contains an *Eco*RI restriction-enzyme site to facilitate cloning.

Total mRNA was isolated from 1–5 mg of muscle tissue (Chomczynski and Sacchi 1987; Muntoni and Strong 1989), and the first-strand cDNA synthesis was carried out using 400 units of Moloney murine leukemia reverse transcriptase according to a method described elsewhere (Bulman et al. 1991*a*). cDNA from normal and deletion cases was subjected to PCR using dimethylsulfoxide (DMSO) buffer (Kogan et al. 1987) and *Taq* polymerase (Cetus). Amplified products were separated on 1.5% agarose gel, and the gel was blotted onto GeneScreen+™ and was probed with a cloned cDNA or an internal oligonucleotide primer. For large PCR products generated with the 1F and 15R primer set, end-labeled 15R primer was added in the PCR reaction mixture to increase the sensitivity of detection. In this case, amplified products from 30 cycles were separated on 1.2% agarose gels and were dried and autoradiographed.

Gel-purified amplified products were either cloned in Bluescript and sequenced using T3, T7, and PCR primers by the dideoxy sequencing method (Sanger et al. 1977) or sequenced directly using Sequenase (USB) and DMSO buffer according to a method described elsewhere (Winship 1989).

Results

Dystrophin Analysis

Western blot analysis of seven cases with a deletion of exons 3–7 indicated that they all had dystrophin of an estimated size of 380 kD (fig. 1). The levels of dystrophin estimated by densitometric analysis were approximately 12%–15% of that found in normal adult muscle. Identical results were obtained using two different polyclonal antibodies—(1) anti-60K, recognizing amino acids 407–815, and (2) P6, recognizing amino acids 2814–3028. A muscle sample from individual UHS1323 was analyzed using the 1461 antibody alone, but, because of the limited amount of tissue, it was not possible to do quantitative estimation of dystrophin for this individual.

To determine whether the dystrophin of reduced size was correctly localized at the sarcolemma, muscle sections from three BMD individuals (H3924, H3975, and UHH.DF) and from one intermediate/BMD individual (UHS1323) were immunolabeled with the amino-terminal (9219) and carboxy-terminal (1461) antibodies (fig. 2). In three of the samples examined (H3975, UHH.DF, and UHS1323), a large proportion of fibers displaying uniform peripheral staining were observed; however, a few dystrophin-negative fibers were also present. Although the staining intensity was quite variable, in general it was lower than normal. All fibers from case H3924 (fig. 2, panel 2) were dystrophin positive, with staining intensity similar to that of normal fiber. However, as the biopsy from this individual consisted mainly of connective tissue, only about 150 muscle fibers, all necrotic, were scored for each antibody. The presence of dystrophin at its correct localization in the muscle of patients helps to explain the mild phenotype and suggests that transcriptional or posttranscriptional mechanisms must account for the production of dystrophin in BMD patients with a deletion of exons 3–7.

Transcript Analysis

Alternative splicing has been considered to be the most likely explanation for the presence of dystrophin in these deletion patients, as splicing out either exon

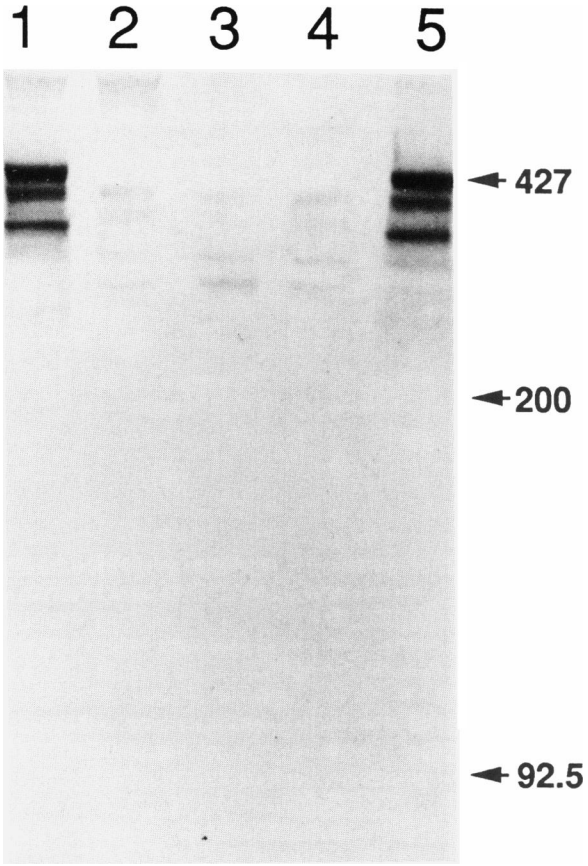

Figure 1 Western blot analysis using antidystrophin antisera (P6) recognizing amino acids 2814–3028. Lanes 1 and 5, Normal human skeletal muscle samples used as controls. Lanes 2–4, Patient samples H4049, H3975, and H4058, respectively. A truncated protein with an estimated molecular mass of 380 kD (the uppermost dystrophin band) was observed. Identical results were obtained using the rabbit anti-60K dystrophin antibodies. Similar results were also obtained for H1456, H3924, UHH.DF, and UHS1323 (for the H3924 and UHH.DF dystrophin immunoblot analysis, only the anti-60K antibodies were used; and, for UHS1323, only the carboxy-terminal antibody 1461 was used, according to a method described elsewhere [Bulman et al. 1991*b*]).

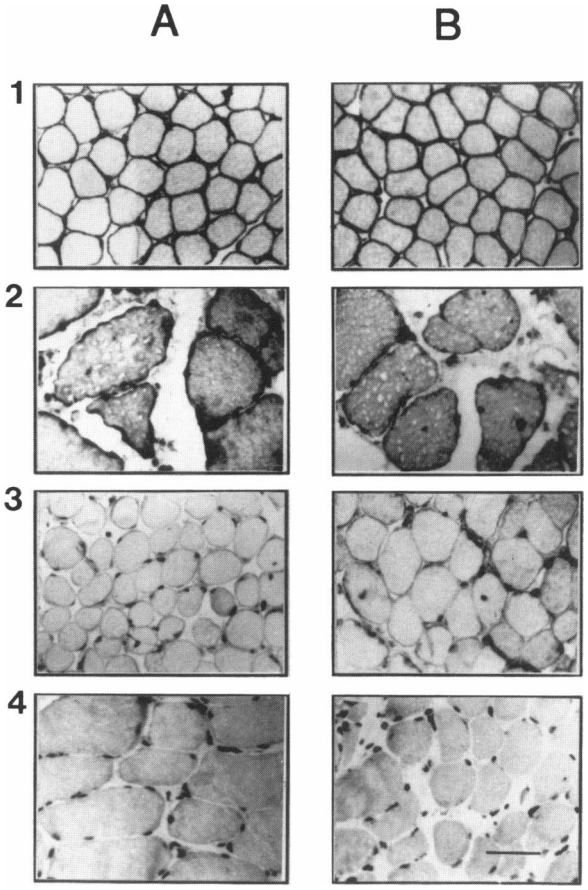

Figure 2 Immunocytochemical analysis of muscle sections, using the amino-terminal (i.e., 9219) antibody (*A*) and the carboxy-terminal (i.e., 1461) antibody (*B*). Panel 1, Normal muscle. Panel 2, H3924 muscle. Panel 3, UHH.DF muscle. Lane 4, DMD patient, dystrophin negative by immunoblot analysis. Scale bar = 10 μm.

2 or exon 8 in the cases with a deletion of exons 3–7 would result in an mRNA with an unaltered reading frame (Chelly et al. 1990; Arahata et al. 1991). Indeed, there is extensive alternative splicing in the mRNAs of a number of muscle-specific genes (Smith et al. 1989), including the dystrophin mRNA (Feener et al. 1989). We have used reverse transcriptase and PCR to examine splicing products from seven patients with a deletion of exons 3–7, all of whom display a mild phenotype. In initial experiments, cDNA was prepared from a primer (10R) in exon 10, and amplification utilized primers in exons 1 and 9. The predicted size of the amplified product is 892 bp from normal dystrophin mRNA and 336 bp if exon 2 is spliced to exon 8. Alternative splicing of exon 1 to exon 8 or of exon 2 to exon 9 gives PCR products of 274 or 154 bp, respectively. Neither of these alternative splice patterns would shift the reading frame of the message.

As shown in the top left panel of figure 3, a 336-bp exon 2–8 splice product was observed in all patients. In normal individuals the 892-bp band corresponding to the intact mRNA was observed. No bands corresponding to an exon 1–8 splice or to an exon 2–9

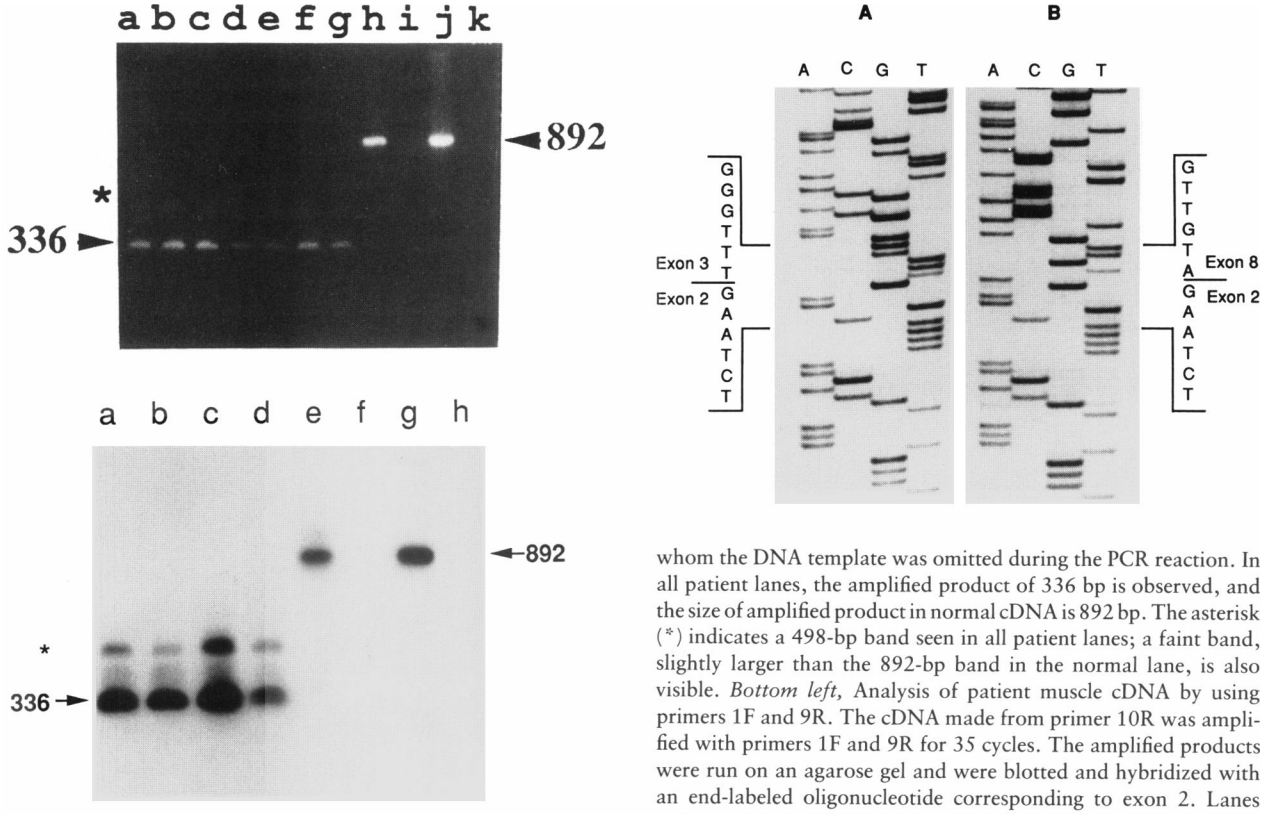

Figure 3 *Top left,* PCR analysis of patient cDNA. The cDNA made from primer 10R was amplified with primers 1F and 9R for 35 cycles. Lanes a–g, Amplified cDNA from deletion cases H3975, H4049, H4058, H3924, UHH.DF, H1456, and UHS1323, respectively. Lane h, Amplified cDNA from a normal individual. Lane i, Reverse-transcription control, the reverse transcriptase omitted during the cDNA synthesis of normal RNA. Lane j, Amplified DNA from 1 pg of control plasmid containing dystrophin cDNA (Malhotra et al. 1988). Lane k, Negative control in whom the DNA template was omitted during the PCR reaction. In all patient lanes, the amplified product of 336 bp is observed, and the size of amplified product in normal cDNA is 892 bp. The asterisk (*) indicates a 498-bp band seen in all patient lanes; a faint band, slightly larger than the 892-bp band in the normal lane, is also visible. *Bottom left,* Analysis of patient muscle cDNA by using primers 1F and 9R. The cDNA made from primer 10R was amplified with primers 1F and 9R for 35 cycles. The amplified products were run on an agarose gel and were blotted and hybridized with an end-labeled oligonucleotide corresponding to exon 2. Lanes a–d, Amplified cDNA from deletion cases H3975, H4049, H4058, and H3924, respectively. Lane e, Amplified cDNA from a normal individual. Lane f, Reverse-transcription control (reverse transcriptase omitted during the cDNA synthesis from normal RNA). Lane g, Amplified DNA from 1 pg of control plasmid containing dystrophin cDNA. Lane h, Negative control in whom the DNA template was omitted during the PCR reaction. The asterisk (*) indicates a 498-bp band seen in patient lanes. *Above,* Direct sequencing of PCR-amplified product by using 1F primer. *A,* Normal individual. *B,* Patient UHH.DF.

splice were observed. Similar results were obtained when the PCR products were assayed by Southern blot analysis with radiolabeled cDNA or with exon-specific probes for exon 2 or exon 8 (fig. 3, bottom left panel). The precise splicing of exon 2 to exon 8 in the 336-bp amplified fragment was verified by sequencing of cloned PCR products, as well as by direct sequencing of the amplified products. The sequence (fig. 3, top right panel) confirmed that the 3′ end of exon 2 was joined directly to the 5′ end of exon 8.

In addition to the expected 336- and 892-bp products, a minor band, slightly larger than the major product, was seen in all samples (fig. 3, top left and bottom left panels). To determine the origin of this 498-bp fragment from patient DNA (denoted by an asterisk in the top left and bottom panels of fig. 3), amplified DNA was eluted from an agarose gel and was reamplified and sequenced directly. This DNA fragment contained a 162-bp sequence of unknown origin, spliced between exons 1 and 2 of the dystrophin transcript; and it contained stop codons in all three reading frames. Southern blots of human genomic DNA probed with this insert gave a smear pattern, indicating its repetitive nature. The inserted DNA is likely from intron 1 and is likely due to aberrant splicing; however, because of its repetitive nature, it was not possible to confirm the origin of this DNA by Southern blot analysis.

Other alternatively spliced transcripts, such as exon 2 spliced to exon 10, 11, 13, 14, or 15, would also restore the translational reading frame, and such mRNA(s) would have been undetected by the primer sets chosen above. In view of the immunoreactivity of patient dystrophin to antiserum raised against exons 4–16 of the DMD gene, the 3′ end of the spliced-out region could not extend beyond exon 15. In order to investigate the possible presence of one of these species of dystrophin mRNA, a longer cDNA synthesized from a primer in exon 16 was amplified with primers from exons 1 and 15 (fig. 4). The major amplified product in each patient was 1.21 kb in size, the size expected from splicing of exons 2–8. In case H3975, a smaller-sized product (1.08 kb) was also amplified (fig. 4, lane b). The 1.08-kb DNA was eluted from the agarose gel and was reamplified, cloned, and sequenced. Of three different clones sequenced, two were missing exon 9, as well as exons 3–7; in the third clone, exon 9 was present, but exon 12, as well as exons 3–7, were absent. Deletions of exons 9 and 12 are possibly due to missplicing events. Missplicing, both that which is deletion induced and that which occurs in normal muscle, has been reported in the DMD gene (Chelly et al. 1990); however, the large extent of missplicing in H3975 mRNA is not yet explainable. In any event, a deletion of either exon 9 or exon 12, when accompanied by a deletion of exons 3–7, does not restore the translational reading frame and therefore cannot explain the mild phenotype in this individual. Thus, in two sets of experiments we have been unable to detect alternatively spliced mRNA that could explain the presence of dystrophin in the mild cases.

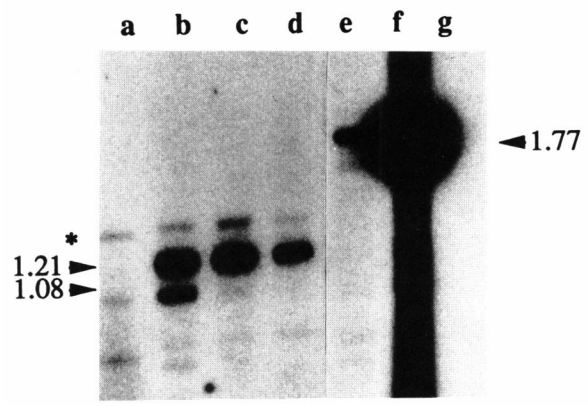

Figure 4 Analysis of patient muscle cDNA by using primers 1F and 15R primers. Lane a, fX *Hae*III marker. Lanes b–d, Amplified cDNAs from cases H3975, H4049, and H4058, respectively. Lane e, cDNA from a normal individual. Lane f, Amplified DNA from 1 pg of plasmid containing the dystrophin cDNA. Lane g, Negative control in whom PCR reaction was carried out without any DNA template. In lanes b–d, a 1.21-kb major amplified product is observed. In lane b, a 1.08-kb band is also present. The expected normal size product (lanes e and f) is 1.77 kb. The asterisk (*) indicates a 1.35-kb band present in all patients, presumably containing the aberrantly spliced 162-bp sequence from intron 1.

RNA Editing

In the patients discussed above, deletion or insertion of nucleotides in the mature mRNA (RNA editing) could also bring the reading frame back into register. However, RNA editing would have to restore the reading frame before a stop codon was encountered in exon 8. Sequencing of PCR-amplified products did not reveal any sequence changes, ruling out RNA editing as the possible mechanism to restore the reading frame in these mildly affected individuals.

Deletion-Endpoint Mapping

It is possible that a deletion-induced cryptic promoter in intron 2 or intron 7 could be responsible for initiating synthesis of mRNA that would not have amplified in the experiments described above. In several genes, downstream promoters have been described; for example, the two different isotypes for alkali myosin light-chain $MLC1_F$ and $MLC3_F$ are encoded by a single locus that has two distinct promoters and gives rise to two different transcripts (Daubas et al. 1985). Introns 2 and 7 in the dystrophin gene are very large (Malhotra et al. 1988; Boyce et al. 1991) and could contain promoter-like elements. To investigate this possibility, deletion breakpoints were mapped. The availability of a large number of probes throughout intron 7 (the XJ region) (Ray et al. 1985; Thompson et al. 1986) has allowed us to map the 3′ deletion breakpoint in 11 individuals with a deletion of exons 3–7. As shown in figure 5, the deletion endpoints of both BMD and intermediate patients are scattered throughout intron 7, and that of one severe patient is internal to the breakpoints in mild patients. The lack of correlation between the 3′ endpoint and severity of phenotype argues against the presence of an intron 7 promoter-like element that is activated by the deletion. It is still a possibility that such elements exist in intron 2, and in that case the putative promoter element(s) is expected to initiate transcription at an unidentified exon contained in intron 2. Such an exon would not be included in the normal dystrophin mRNA.

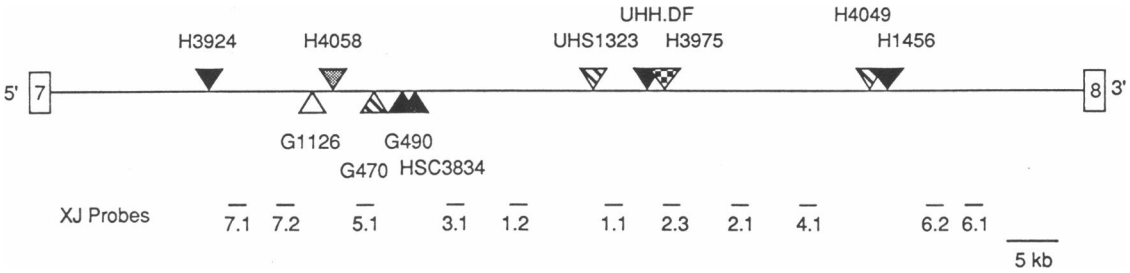

Figure 5 Deletion breakpoints in intron 7. Triangles above the line indicate patients examined in the present report. For those below the line, muscle biopsies were unavailable. Blackened triangles denote BMD cases; diagonally striped triangles denote intermediate/BMD cases; the gray triangle denotes the intermediate case; the checkered triangle denotes the ?BMD case; and the unmarked triangle denotes the DMD case. Overlapping triangles indicate that the breakpoint positions are not defined with respect to each other. The clinical details for G470, G490, and G1126 have been described elsewhere (Malhotra et al. 1988). HSC 3834 is a familial 7-year-old mild case (his uncle, who had a similar deletion, was able to walk independently, without braces, after the age of 30 years). Numbers beneath the bars indicate the position of XJ probes, and boxes indicate exons 7 and 8. A scale bar is given in the lower right corner.

Discussion

One potential explanation for the mild phenotype in apparent frameshift individuals was that in some patients the deleterious effect of the lack of dystrophin is mitigated by some unknown factor. Indeed, mdx mice, lacking dystrophin, do not display a severe phenotype (Sicinski et al. 1989). It was, therefore, essential to establish whether dystrophin was being produced in these mild cases with a deletion of exons 3–7. The western blot analysis revealed that dystrophin, though less abundant and slightly reduced in size, was present in patients displaying a mild phenotype with a deletion of exons 3–7. The immunocytochemical analysis revealed that it was correctly localized in the sarcolemma. This obviated the need to postulate the involvement of secondary factors and suggested that transcriptional or posttranscriptional mechanisms must account for the production of dystrophin in BMD patients with a deletion of exons 3–7.

The transcript analysis did not identify an in-frame transcript in these BMD patients, indicating that alternative splicing could not be documented to account for the presence of dystrophin in these mild cases. Our results are in apparent contradiction with those of Chelly et al. (1990), who found that, in two BMD patients with a deletion of exons 3–7, in-frame alternatively spliced mRNA (exon 1 spliced to exon 8 and exon 2 spliced to exon 10) existed at levels of 1%–2% of the amount of out-of-frame mRNA. In our own experiments, PCR analysis from a serial dilution of patient cDNA indicated that we could have detected message present at 1%–2% of the level of the exon 2–8 splice product. Also, the fact that we detected aberrant splicing makes it unlikely that any substantial amount of alternative splicing was missed in our experiments. The biological significance of the low level of in-frame transcripts described by Chelly et al. (1990) is unclear, although it is possible that this level of mRNA might account for the 12%–15% of dystrophin, if translation was up-regulated in response to the dystrophin deficiency.

Arahata et al. (1991) have described two cases with a deletion of exons 3–7 in whom muscle sections stain negatively with antibodies raised against a synthetic peptide from exon 8 and stain positively with antibodies raised against a peptide from exons 13/14. A negative result with exon 8–specific antibody is difficult to interpret, as it is likely that the configuration of dystrophin produced in these patients would be disrupted and that epitopes recognized by the antisera could be altered.

Other mechanisms that might be involved in the production of dystrophin in the deletion patients include RNA editing, the presence of a cryptic promoter, reinitiation, and ribosomal frameshifting. RNA editing has been observed in trypanosomes (Benne 1990) and mammalian apolipoprotein-B RNA (Chen et al. 1990), as well as in plant mtRNAs (Covello and Gray 1989). Sequencing of amplified RNA from individuals with a deletion of exons 3–7 in the dystrophin gene did not reveal any sequence changes, suggesting that extensive RNA editing is unlikely to be the mechanism for reestablishing the translational frame. Also, a new mRNA synthesized from a cryptic promoter in intron 7 seems highly unlikely, given the scattered distribution of deletion endpoints in the intron. A cryptic promoter in intron 2 remains a formal possibility, since

the probes were not available to map the deletion endpoints in this intron.

In a number of biological systems, secondary reading frames are used for coordinate expression of proteins. In these systems ribosomal frameshifting or reinitiation of protein synthesis on internal AUG codons allows access to alternative reading frames (Cattaneo 1989). In an earlier study, we have described an internal AUG with a Kozak consensus sequence in exon 8 of the dystrophin gene, a situation that could allow the translation of a shortened dystrophin molecule beginning at amino acid 253 (Malhotra et al. 1988). Such a protein would be predicted to have a molecular weight of 398 kD, similar in size to the dystrophin found in these patients (fig. 1). Ribosomal frameshifting, on the other hand, would allow translation of dystrophin from the first AUG in exon 1 and would restore the frame around the deletion junction in exon 2 or exon 8. Such a protein would be predicted to have an approximate size of 405 kD, not distinguishable from the 398 kD predicted by the reinitiation model. The experiments described here cannot exclude, or distinguish between, these possibilities.

There are a number of other deletions that are confined to the first 10 exons of the dystrophin gene and that follow the reading-frame rule. Deletions of exons 3 and 3–4 are in-frame events, and, as expected, individuals with these deletions have the mild phenotype (Koenig et al. 1989). On the other hand, deletions of exons 3–6, 4–7, 5–7, and 8–9 are expected to cause a shift in the translational frame, and these individuals have the severe phenotype (Malhotra et al. 1988). Therefore, at the 5′ end of the gene, with deletions confined to the first 10 exons, the exceptions to the reading-frame rule are limited only to the deletions of exons 3–7. It is possible that, because of the involvement of large introns (introns 2 and 7) at both deletion breakpoints, the topology of the gene with this deletion is different from that of other deletions at the 5′ end of the gene. It is also possible that different transcriptional and posttranscriptional mechanisms could be operative in restoring the translational reading frame, and this could explain the variation in phenotype in individuals with this deletion. Unfortunately, at present there are not any reports of dystrophin studies on the severe (i.e., DMD) patients with a deletion of exons 3–7, and we were unable to obtain biopsies from such individuals. Such studies are essential for understanding the variable phenotype in patients with this deletion.

In conclusion, we found dystrophin in BMD and intermediate patients with a frameshift deletion of exons 3–7. We did not find any evidence for alternative splicing and RNA editing as operative mechanisms in restoring the reading frame in these patients. Mapping of 3′ deletion endpoints in intron 7 argues against activation of a cryptic promoter in this intron. Yet, the translational reading frame is restored by a transcriptional or posttranscriptional mechanism. Reinitiation of protein synthesis from the AUG in exon 8 is one possibility, while ribosomal frameshifting is another possibility, for restoring the proper reading frame. The knowledge of the exact position of deletion breakpoints, and the use of exon-specific antibodies, may make it possible to determine the mechanism(s) involved in restoration of reading frame in these deletion patients. It is also likely that, depending on the nature of the deletion breakpoints, different mechanisms could be involved in different patients, as the intronic sequences could play important roles.

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